

In the Claims

1-47 (canceled).

48 (currently amended). A method for the identification or cloning of polynucleotides encoding a selected phenotype, the method comprising (i) cloning environmental DNA fragments into *E. coli* cloning vectors and propagating said vectors in *E. coli* to produce a metagenomic library in *E. coli* cells, (ii) identifying or selecting cloning vectors in said library which contain DNA fragments having a particular characteristic of interest, (iii) inserting a target polynucleotide construct into said identified or selected cloning vectors in a region of said identified or selected cloning vectors distinct from the DNA fragments having a particular characteristic of interest, wherein said target polynucleotide construct comprises a nucleic acid encoding ~~a functional~~ an origin of transfer functional in *E. coli* and a nucleic acid encoding an integrase functional in a selected recipient host cell having a genome distinct from *E. coli*, thereby conferring to the modified cloning vectors the ability to be transferred into said selected recipient host cell, (iv) transferring the modified cloning vectors into said selected recipient host cell and integrating said modified cloning vectors and/or the DNA fragments having a particular characteristic of interest which they contain into the genome of said selected recipient host cell and (v) identifying or cloning the DNA fragments contained in said modified cloning vectors which encode said selected phenotype in said selected recipient host cell,

wherein ~~said environmental DNA fragments are cloned in *E. coli*~~ and said selected recipient host cell is a microorganism or host cell other than *E. coli*.

49 (previously presented). The method of claim 48, wherein the cloning vectors are selected from the group consisting of a cosmid, a fosmid, P1 and BAC vectors.

50 (previously presented). The method of claim 48, wherein the library comprises a plurality of unknown polynucleotides.

51 (previously presented). The method of claim 48, wherein the library comprises a plurality of environmental DNA fragments.

52 (previously presented). The method of claim 48, wherein said modified cloning vectors are integrated into the genome of the selected recipient host cell by site-specific integration.

53 (canceled).

54 (currently amended). The method of ~~claim 53~~ claim 48, wherein the origin of transfer is an origin of transfer contained in a plasmid selected from the group consisting of RP4, pTiC58, F, RSF1010 and R6K(α).

55 (previously presented). The method of claim 48, wherein the integrase is Φ C31 integrase.

56 (previously presented). The method of claim 48, wherein the target polynucleotide construct comprises a transcriptional promoter functional in the recipient selected host cell.

57 (previously presented). The method of claim 48, wherein the target polynucleotide construct is contained in a transposable nucleic acid construct.

58 (previously presented). The method of claim 57, wherein the transposable nucleic acid comprises two inverted repeats, the target polynucleotide construct and a marker gene, said inverted repeats flanking the target polynucleotide construct and the marker gene.

59 (previously presented). The method of claim 48, wherein the cloning vectors comprise a first marker gene and wherein, in step ii), the selected cloning vectors are modified by:

contacting in vitro, in the presence of a transposase, the selected cloning vectors with a transposon comprising two inverted repeats, the target polynucleotide construct and a second marker

gene distinct from the first marker gene, with inverted repeats flanking the target polynucleotide construct and the second marker gene, and

selecting the cloning vectors which have acquired the second marker gene and which have lost the first marker gene.

60 (previously presented). The method of claim 48, wherein, in step (i), the cloning vectors which contain a polynucleotide having a particular characteristic are selected by molecular screening.

61 (previously presented). The method of claim 48, wherein, in step (iii), the modified cloning vectors are transferred into the selected recipient host cell by conjugative transfer.

62 (previously presented). The method of claim 48, wherein said particular characteristic is a nucleic acid sequence or motif characteristic of a particular activity or gene.

63 (currently amended). The method of claim 48, wherein said environmental DNA fragment is at least 10 kilobases in length.

64 (previously presented). The method of claim 48, wherein said environmental DNA fragment is between about 40 kilobases and 80 kilobases in length.

65 (previously presented). The method of claim 52, wherein said site specific integration into the genome of the recipient host cell is stable.

66 (canceled).

67 (new). A method for the identification or cloning of polynucleotides encoding a selected phenotype, the method comprising:

- (i) cloning environmental DNA fragments into *E. coli* cloning vectors and propagating said cloned environmental DNA fragments in *E. coli* to produce a metagenomic library;
 - (ii) identifying or selecting cloning vectors in said library which contain DNA fragments having a particular characteristic of interest or encoding a selected phenotype;
 - (iii) inserting a target polynucleotide construct into said identified or selected cloning vectors in a region of said identified or selected cloning vectors distinct from the DNA fragments having a particular characteristic of interest to form a modified cloning vector, wherein said target polynucleotide construct comprises a nucleic acid encoding an origin of transfer functional in *E. coli* and a nucleic acid encoding an integrase functional in a selected recipient host cell having a genome distinct from *E. coli*, thereby conferring to the modified cloning vectors the ability to be transferred into said selected recipient host cell;
 - (iv) transferring the modified cloning vectors into said selected recipient host cell and integrating said modified cloning vectors and/or the DNA fragments having a particular characteristic of interest which they contain into the genome of said selected recipient host cell; and
 - (v) identifying or cloning the DNA fragments contained in said modified cloning vectors which encode said particular characteristic of interest in said selected recipient host cell,
- wherein said selected recipient host cell is a microorganism or host cell other than *E. coli*.

68 (new). The method according to claim 67, wherein said identifying or selecting a particular characteristic of interest comprises sequencing said DNA fragments after cloning to identify a consensus sequence or a motif associated with said particular characteristic of interest.

69 (new). The method according to claim 67, wherein said identifying or selecting a particular characteristic of interest comprises hybridizing said DNA fragments after cloning with probes that identify a consensus sequence or a motif associated with said particular characteristic of interest.

70 (new). The method according to claim 67, wherein said identifying or selecting a particular characteristic of interest comprises amplifying said DNA fragments after cloning with

primers that identify a consensus sequence or a motif associated with said particular characteristic of interest.